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Characterization of a novel lyophilized chitosan hydrogel complex for the controlled release of a highly water soluble drug, niacinamide

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Abstract

The purpose of this research was to prepare and characterize a novel lyophilized chitosan-based hydrogel complex (termed CS-M) for controlled drug delivery applications using a highly water soluble model drug, niacinamide. Characterization studies were undertaken to evaluate the physical-chemical properties, polymer swelling, *in vitro* controlled release kinetics, tissue bioadhesion, intestinal permeability and stability of the novel chitosan complex. Additionally, a comparative analysis was conducted with commercial polymers namely, Carbopol 974P-NF® and hydroxypropyl methylcellulose (HPMC K4M). FT–IR and ^{1}H NMR studies confirmed that despite alteration in physical structure and morphology of the chitosan complex the chemical properties remained unchanged, when compared to the parent chitosan compound. Polymer swelling studies showed consistency in the structural integrity and water uptake of CS-M compared to other polymers which showed inconsistent swelling and disintegration behavior over a 5 h period. *In vitro* controlled release profiles of CS-M showed a slower, more controlled release rate of niacinamide than other polymers indicating the influence of polymer swelling capacity on water uptake and subsequent drug release. CS-M demonstrated an overall increase in bioadhesion to intestinal tissue when compared to commercial polymers at same concentrations. Similarly, drug transport through everted sac intestinal tissue showed enhanced absorption properties of CS-M when compared to other polymers. Finally, a 3 month accelerated stability study showed all polymers including CS-M to be stable when formulated with niacinamide. Overall, the modified chitosan-based hydrogel polymer, CS-M, demonstrated enhanced characteristics indicating its potential to be used as a controlled release excipient in oral drug formulations.

Keywords: Chitosan hydrogel complex, controlled drug delivery, niacinamide, lyophilization, Carbopol 974P-NF, HPMC K4M

Introduction

Chitosan, an amino-polysaccharide derived by deacetylation from parent compound chitin, is abundantly found on the exoskeleton of crabs. In recent times, it has been widely applied in the fields of drug delivery and tissue engineering because of its biodegradability, non-toxicity and good biocompatibility (1-4). It is presently approved as a food additive in Japan, Italy and Finland (5). However, there are some limitations to the use of CS such as poor solubility in aqueous medium (at higher pH), poor compressibility and erratic flow properties that make it difficult to incorporate this polymer in preparation of controlled release dosage forms. CS does, however, make salts with inorganic and organic acids such as hydrochloric acid, acetic acid, glutamic acid, and lactic acid. These salts are soluble in water, the solubility depending on the degree of deacetylation and the pH of solution. To further enhance the solubility of this polymer and to improve its mucoadhesive and permeation enhancing properties, various derivatives such as chitosan glutamate, N-trimethyl chitosan chloride and thiolated chitosans have been developed (6-8). However, these processes require extensive chemical treatment and processing steps which in turn lead to increased expense and time.

The objective of our study was to develop a modified chitosan hydrogel complex (termed CS-M) designed

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to overcome these limitations and serve as an ideal controlled release agent in oral dosage forms. The formation of CS-M polymer was based on a simple freeze-dried process that resulted in a compound with improved qualities than that of its parent compound, chitosan. Additionally, when compared to commercially available polymers, the performance of the chitosan hydrogel was vastly improved. Studies were conducted to characterize the CS-M polymer using physical-chemical methods such as Fourier transform infrared (FT–IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Polymer swelling studies were conducted to determine the influence of swollen CS-M polymers on the controlled release characteristics of niacinamide. The swelling behavior was compared with conventional rate controlling polymers, hydroxypropyl methylcellulose (HPMC K4M) and a carbomer (Carbopol 974P-NF®), and correlated with the *in vitro* drug release characteristics of these polymers. Intestinal tissue bioadhesion and drug permeability were also evaluated to gauge the effects of the hydrogel polymer on retention of the dosage form on the tissue and its subsequent effect on transport of the drug through rat small intestine. Finally, accelerated stability studies were conducted to determine niacinamide drug degradation upon storage for prolonged periods of time in all of the polymer formulations.

Materials and methods

Materials

Chitosan (CS, 50 K Da, low molecular weight) and the model drug, niacinamide, were purchased from Sigma-Aldrich (St. Louis, Missouri). Avicel® pH 101 (microcrystalline cellulose) was provided by FMC Biopolymer (Newark, Delaware). Methocel® K4M, hydroxypropyl methylcellulose (HPMC K4M) was provided by Dow Chemicals (Midland, Michigan), Carbopol 974P-NF® was provided by Noveon (Cleveland, Ohio). Magnesium stearate and talc was purchased from Fisher Scientific (Fairlawn, New Jersey). All chemicals and solvents were of analytical grade.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of chitosan (CS) and CS-M using a Nicolet Magna-IR 560 (Nicolet Instrument Technologies, Madison, WI) were recorded. A 1% sample of CS and CS-M was mixed in powdered potassium bromide and then cold compressed in a 0.5 in (inside diameter) die under 200 MPa pressure. The cold compressed sample-potassium bromide discs were transparent and spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} using a 128 scan.

Proton Nuclear Magnetic Resonance (^{1}H-NMR)

NMR spectra for CS and CS-M were obtained on a Varian Gemini 200 MHz broadband (Varian, Palo Alto, CA) spectrophotometer. The reference used was 3-triisopropylsilyl propylsulfonate. 5 mg samples were diluted in 0.75 ml of solvent, 1% DCL/D_2O and study performed at room temperature.

Preparation of the modified chitosan hydrogel complex formulation (CS-M)

1g of low molecular weight (50 KDa) chitosan flakes was dissolved in a 1% acetic acid solution. Subsequently, sodium chloride was added in 1:4 ratio into the mixture. The solution was filtered to remove any undissolved particles, poured into a vacuum flask, sealed and placed into the shell freezer (Labconco, Kansas City, Missouri) for 1 h. The frozen sample was transferred to a freeze-dry system, Freezone® 4.5 (Labconco, Kansas City, Missouri), and dried overnight (18h). The resulting product was retrieved as a free flowing (angle of repose, $< 35^o$), fine white powder form of CS-M with excellent compressibility as tested using a single punch tablet machine (Carver Inc., IN).

Preparation of CS-M polymer-based formulations

Multiple tablet formulations were prepared as follows: Formulations (F1 – F3) contained 20% concentrations of each polymer HPMC K4M, Carbopol 974P-NF 97® and CS-M, respectively, within the formulations (Table 1). Niacinamide, a highly water soluble drug, was used as a model drug in these formulations. Polymer swelling capacity, i*n vitro* dissolution, tissue bioadhesion, drug transport and stability studies were carried out to assess characteristics of the newly developed CS-M hydrogel polymer. Avicel® pH 101 (microcrystalline cellulose) was used as a filler and magnesium stearate and talc as a lubricant and glidant, respectively. After blending the powders, the formulations were transferred to a 16-station mini tablet rotary press (Riddhi Pharma, India) for direct compression. Tablets without polymers were prepared as controls. All tablets were enteric coated with 5% Eudragit® L–100 to protect the tablet(s) from the simulated gastric fluids. The core tablets were evaluated for weight variation and hardness. All formulations were subject to friability testing on a

VanKel friabilator model 45-2000 (Cary, NC) prior to continuing with experiments.

Polymer swelling studies

Swelling studies of tablets provide information of water uptake by the polymer and its influence on the drug release mechanisms in the G.I. transit system (9). Formulations (F1 – F3) were subjected to swelling studies in a USP Type 1 (basket) dissolution apparatus (Vankel, Cary, NC). Tablets were placed in the basket attached to the dissolution apparatus containing 300 ml of pH 6.8 PBS solution. The water bath was maintained at 37 $\pm$ 0.5 °C and the basket rotating speed was maintained at 50 rpm. At designated time intervals, the basket was removed and excess water was wiped off from the mesh and surrounding area. The swollen tablet weight was recorded and the water uptake determined by the following equation (9):

$$Q = \frac{(W_s - W_d)}{W_d} \times 100$$

where,

Q = water uptake (%), W_d = weight of dry tablet and W_s = weight of swollen tablet. All swelling studies were performed in triplicate.

***In vitro* drug release studies**

Dissolution studies were conducted using the USP Type I (Basket) apparatus for all formulations (F1 – F3). The basket speed rotation was maintained at 50 rpm and the temperature was set at 37 $\pm$ 0.5 ºC. The drug release studies were conducted in 300 ml of 0.1N HCl, for two hours, to mimic the gastric environment, replaced with 300 ml of phosphate buffered saline (PBS) solution at a pH of 6.8 for an additional 5 h. At predetermined time intervals, 3 ml samples were withdrawn and assayed for drug release spectrophotometrically at a wavelength of 262 nm. All experiments were conducted in triplicate.

Evaluation of tissue bioadhesion

The determination of adhesion strength of the polymer samples to the rat intestinal tissue was done using a Du Nouy surface tensiometer model 20 apparatus per a previously described method (10). Following established animal handling protocols and approval from the university animal use committee, intestinal tissue was harvested from female Sprague Dawley rats (Harlan, San Diego, CA) which were anaesthetized for 5 minutes using isofluorane. A foot pinch test was performed prior to cervical dislocation being carried out. Three tablets from each polymer formulation were glued on to a petri-dish. The tablets were soaked for 5 min in a phosphate buffered saline (PBS) medium. A 1.5 cm long segment of rat intestine was cut to expose the mucosal side. The tissue was mounted on a metal ring (4.8 cm length/1.4 cm diameter dimensions) and the mucosal surface was allowed to make contact with tablet for a duration of 2 min. The metal ring was slowly raised until the tension caused the tablet surface to detach from the tissue. The value was then recorded in dynes/cm^2 as observed from the dial.

Table 1: Formulation chart for niacinamide tablet formulations containing different polymers

Ingredients (%)	F1	F2	F3
Avicel pH 101	57.57	57.57	57.57
HPMC K4M	20	-	-
Carbopol 974P-NF	-	20	-
CS-M	-	-	20
Talc	0.5	0.5	0.5
Magnesium Stearate	0.5	0.5	0.5
Niacinamide active	21.43	21.43	21.43
Total (%)	100	100	100

In vitro tissue permeability studies

The everted sac technique was used to determine drug transport across the rat intestine (11). The small intestine segment of the rat was rinsed with 0.9% ice-cold saline and transferred into a beaker filled with oxygenated Tyrode buffer, pH 7.4. Tablets formulations (F1-F3) were incubated in 10 ml Tyrode buffer, in a 16 X 100 mm test tube for 20 minutes, which is the optimal time for the tablet to equilibrate at the swollen state (12). The small intestine was cut into 6 cm segments, everted on a glass rod and tied at one end with a suture thread. The suture was then tied to a small washer and the open end of the intestine was fitted over the tip of a glass pipette with open ends. The open end of the glass pipette was pushed into one of the three openings of a rubber stopper. The intestine was then placed into the test tube containing the tablet. The rubber stopper was secured in an Erlenmeyer flask with 200 ml of water and placed into a moving water bath model 4682 (Lab-Line, Melrose Park, Illinois) at 37 $\pm$ 0.5 °C with a speed of 50 rpm. 1.0 ml of Tyrode buffer was added to the everted intestinal sac and 0.5 ml samples were withdrawn into vials. Samples were taken at designated time intervals and analyzed on the HPLC. All everted sac studies were performed in triplicate. Samples of niacinamide collected from the mucosal

side of the intestinal everted sacs were analyzed on an HP Series 1100. A Prodigy 5µ, ODS3 100 Å, 250mm x 4.60 mm column was used to analyze the drug. The temperature was maintained at 25ºC and samples were measured at 262 nm. The mobile phase consisted of 10 mM KH_2PO_4 and methanol (40:60). The flow rate was set at 1 ml/min. The injection volume was 20 µl and the relative retention time was 4 minutes.

Stability studies

All formulations were subjected to accelerated stability testing, by placing each of the tablet formulations in the stability chamber, model 9005 (VWR, Cornelius, Oregon) maintained at 40 ºC with a relative humidity of 75%. Samples were taken out at one, two and three month intervals and dissolution testing was performed on these samples. The samples were analyzed at a wavelength of 262 nm using a UV-Vis spectrophotometer (Shimadzu, CA).

Results and discussion

Characterization of CS-M: FTIR and ^{1}H-NMR spectroscopy

The FT-IR spectra of the investigated polymer are represented in Figure 1. Figure 1 (a) shows the absorption peaks of CS-M, whereas, Figure 1 (b) and 1 (c) show the absorption peaks of sodium chloride and untreated chitosan, respectively. Modified chitosan (CS-M) (Figure 1a) showed a characteristic absorption band at 3420 cm^{-1} attributed to the stretching vibration of the amide (N-H) and the hydroxyl (O-H) groups whereas the width of the band indicated that the hydrogen bonding was enhanced (13-14). The bands at 1600 and 1260 cm^{-1} show the bending vibrations of the N-H and the O-H groups, respectively. In Figure 1c, the absorbance spectrum at 3420 cm^{-1} was much more muted than that of the CS-M. However, the absorbance peaks for both compounds were at similar wavelength numbers indicating that there was no difference in the structure of the two compounds. The peaks ranging from 1640-1740 cm^{-1} represented the carbonyl (C=O) functional group. This functional group was more dominant in CS-M than that of parent chitosan indicating that there was more acetylation in the CS-M structure.

Overall, the objective of FTIR study was to identify the functional groups that exist within both the polymers. From observations of the FTIR spectrum, the chemical structure of CS-M was unaltered from that of the parent compound indicating that the modified version retained all the chemical properties of the parent compound, chitosan (CS).

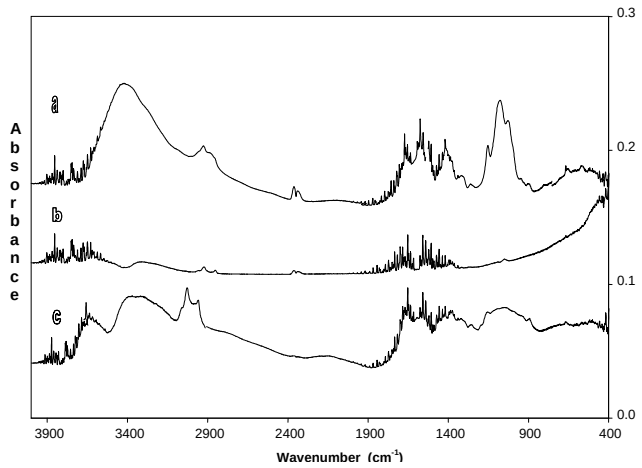

Figure 1. Fourier Transform Infrared (FTIR) data for (a) chitosan hydrogel salt complex (CS-M) (b) sodium chloride, and (c) parent compound, chitosan (CS).

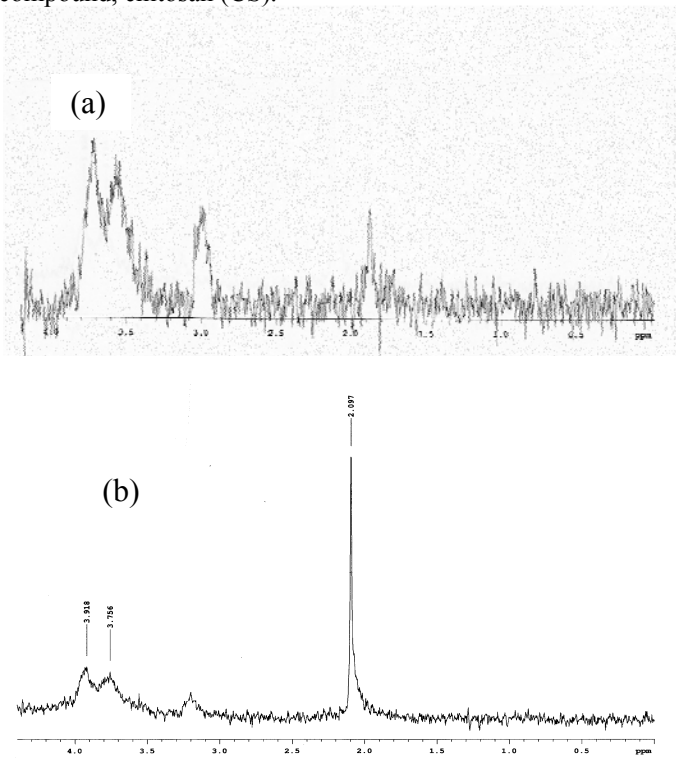

Figure 2. Proton nuclear magnetic resonance (^{1}H-NMR) data for (a) chitosan (CS) and (b) chitosan hydrogel salt complex (CS-M).

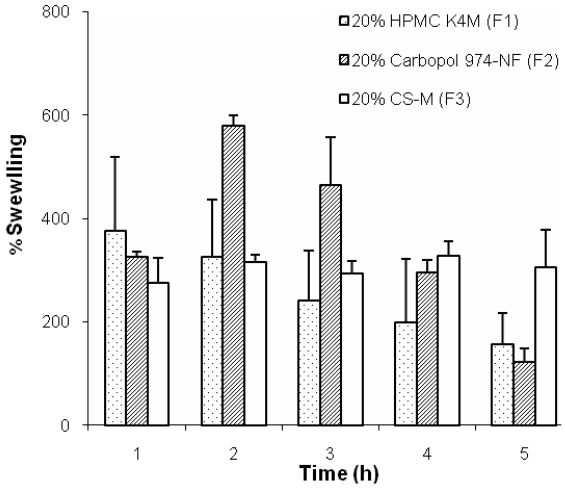

Figure 3. Polymer swelling (water uptake) capacity of 20% concentration of HPMC K4M (F1), Carbopol 974P-NF®(F2) and CS-M (F3) formulations (n=3).

Figures 2(a) and 2(b) represent the ^{1}H NMR spectra of CS and CS-M, respectively. The CS spectra demonstrated a decrease in acetyl group at 2.097ppm compared to the CS-M spectrum (Figure 2b). Other than the sharp distinction of the acetyl group, CS and CS-M spectra were quite similar. The presence of an increased number of acetyl groups in CS-M was further confirmed after the FTIR analysis indicated the same trend. Thus, this study also verified that there was no change in the chemical structure of CS-M when compared to CS.

In vitro polymer swelling studies

Figure 3 compared the amount of water uptake and the resultant swelling effect from formulations (F1, F2 and F3), i.e., 20% HPMC K4M, 20% Carbopol 974P-NF® and 20% CS-M polymer formulations, respectively. As represented in Figure 3, for formulation (F3) containing 20% CS-M, the amount of water uptake reached equilibrium within one hour of the run whereby swelling increased by ~276%. After 5 h, water uptake only increased to 304%, however the shape and integrity of the tablet was maintained throughout the duration of the study. With formulation (F1) containing 20% HPMC K4M hydration occurred during the first hour with 375% increase in swelling but slowly diminished over the five hour study indicating a slow erosion process. HPMC K4M polymer is known to quickly hydrate as demonstrated in the literature (15), this effect was confirmed in our study. Formulation (F2) containing 20% Carbopol 974P-NF® had a much higher water uptake as expected due to its well documented swelling properties (16). In our study, equilibrium swelling occurred at 2 h, with a 580% increase after which an erosion phenomenon was observed. After 5 h, most of the Carbopol 974P-NF® containing tablet had disintegrated into the surrounding aqueous medium.

Both HPMC K4M and Carbopol 974P-NF® containing formulations demonstrated higher swelling property than CS-M, however in both cases, tablet disintegration and erosion was evident within 5 h. In comparison, CS-M had a relatively lower uptake of water but showed no signs of erosion even after the 5 h study, maintaining consistency and rigidity of its shape and a swelling capacity of 300 times its original weight. It is postulated that the presence of sodium chloride crystals played an important role in the swelling rate of the polymers when compared to the other polymers in the study. The retention of shape could play an important role in the design of controlled release systems with predictable kinetics. The effect of water on the tablet formulation created microscopic pores as sodium chloride slowly dissolved in the surrounding medium. The creation of the pore effect in essence allowed the CS-M polymer to swell rapidly and reach equilibrium within the first hour. The results of the swelling study correlated well with *in vitro* dissolution data thereby confirming the release pattern of drug was proportional to the swelling capacity of the polymer.

In vitro drug release studies

Optimization studies were conducted to determine the ideal concentration range of CS-M to be used in tablet formulations. As observed in Figure 4, niacinamide drug release characteristics were evaluated at 20% concentrations of HPMC K4M (F1) and Carbopol 974P-NF® (F2) and CS-M (F3). At 20% CS-M concentration, the sodium chloride present in CS-M seemingly controls the release of drug and increases the lag time of drug release. The amount of sodium chloride in the tablet causes a salting out effect in which the salt occupies the water molecules making the CS-M polymer less water soluble. Gonzalez-Rodriguez et al., (17) demonstrated that formulations containing sodium chloride showed longer lag times due to the plastic deformation properties; when

compressed, sodium chloride leads to a less porous, more compact network which strictly controls solvent penetration, drug dissolution and release rate. Another study performed by Amiji (18) stated that sodium chloride at different concentrations was added to chitosan solutions (made with acetic acid and hydrochloric acid) to adjust the ionic strength, and since the interactions between chitosan chains occur primarily through the amine groups, increasing the ionic strength of the medium does enhance the hydrophobic association. Thus, the addition of sodium chloride reduces the amount of drug release.

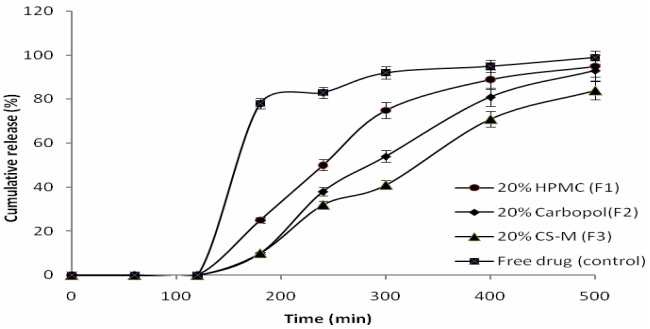

Figure 4 A comparative cumulative release (%) profile of niacinamide drug from 20% concentration of HPMC K4M (F1), Carbopol 974P-NF (F2) and CS-M hydrogel polymer (F3) based formulations (n=3).

The *in vitro* drug release of niacinamide drug from 20% CS-M was compared with similar concentrations of commercially available polymers Carbopol 974P-NF® and hydroxypropyl methylcellulose (HPMC K4M). As observed in Figure 4, the rate of drug release from Carbopol 974P-NF® (F1) and CS-M (F3) was consistent for the first 4 h of the run after which CS-M was observed to be more controlled than the Carbopol 974P-NF® containing formulation for the duration of the study (> 8 h).

HPMC K4M formulation (F1) showed a consistent pattern of release over the duration of the study, however it was observed to release niacinamide at a faster rate than the other two polymer formulations. From the swelling capacity studies, the high water uptake of Carbopol 974P-NF® prolonged the release rate of the drug initially by creating a gel structure and extended channels that prevented the drug from diffusing out of the tablet quickly (19). However, the swelling effect diminished rapidly resulting in a faster release of the drug over the duration of the study. In case of the CS-M containing formulation, a gel transformation occurred which exhibited no disintegration and erosion, maintaining its shape for the duration of the study. This retention of shape ensured steady release of drug and hence prevented dose dumping (20). Overall, CS-M based formulations exhibited an ideal release profile when compared to the commercial brands, thus demonstrating comparable performance to these polymers.

Prior to the in vitro dissolution studies, three tablets were taken from each formulation to verify content uniformity. The total drug content for these tablets averaged 97% $\pm$ 7%.

In vitro bioadhesion studies

The bioadhesion analyses of all three formulations tested are shown on Figure 5. A number of polymeric characteristics are necessary for muco- or bio-adhesion, which can be summarized as follows: (a) strong hydrogen bonding groups (-OH, -COOH) (b) strong anionic charges (c) high molecular weight (d) sufficient chain flexibility (e) surface energy properties favoring spreading onto mucus (21). Figure 5 represents the bioadhesion strength of the various polymers tested (in dynes/cm^2). The strength to break the bond between the tissue and the swollen tablets (fracture theory) were between 31-35 dynes/cm^2. HPMC K4M is a non-ionic polymer, therefore bioadhesion of this polymer to the tissue was probably due to hydrogen bonding.

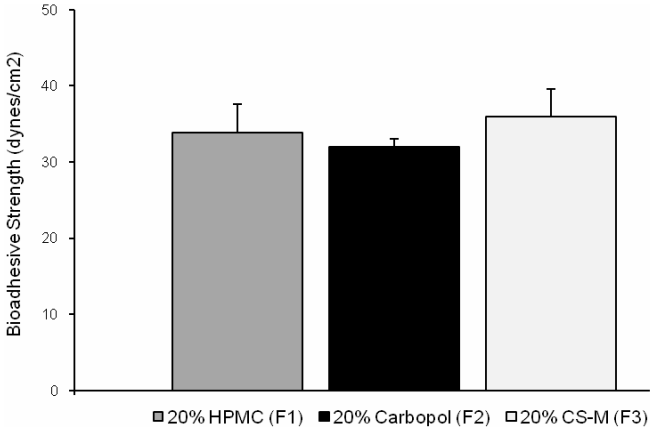

Figure 5 Bioadhesion strength (dynes/cm^2) analysis of tablets containing 20% concentration of HPMC K4M (F1), Carbopol 974P-NF (F2) and CS-M (F3).

Bioadhesion of Carbopol 974P-NF®, an anionic polymer to the intestinal membrane seemed to be because of its partially ionizable carboxylic acid groups. The bioadhesion phenomena of Carbopol 974P-NF® and the membrane tissue composed of two mechanisms, a chemical interaction between functional groups and an interpenetration of the polyacrylic acid chains into the mucus, which was favored by high swelling (12).

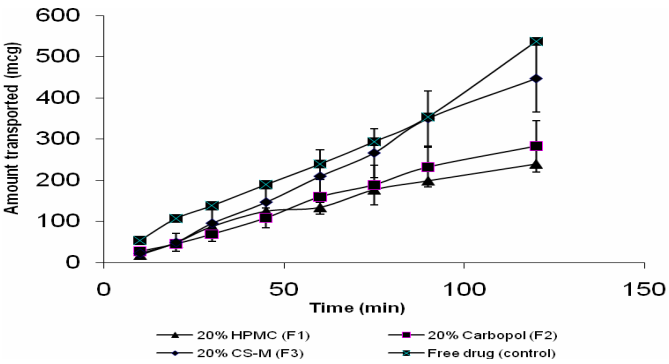

Figure 6 Permeability studies using the intestinal everted sac method showing amount of drug transported from formulations containing 20% concentration of HPMC K4M (F1), Carbopol 974P-NF (F2) and CS-M (F3).

In case of the CS-M formulation, since the parent compound chitosan is a cationic polymer, bioadhesion of CS-M to the intestinal tissue was through the electrostatic interaction of the positively charged polymer with the negatively charged surface of the membrane. Lehr et al., (21), reported that positively charged polymeric hydrogels could possibly develop additional molecular attraction forces by electrostatic interactions with negatively charged mucosal surfaces. Overall, there was no evidence of the bioadhesive strengths of each of the polymer to be significantly different from each other, although CS-M formulations did exhibit a higher bioadhesive force compared to the other polymers.

Drug permeability studies

The permeability of the niacinamide drug was evaluated using the everted sac intestinal tissue method (Figure 6). The total amount of drug transport for the various formulations were as follows: CS-M > Carbopol 974P-NF > HPMC K4M . CS-M showed the highest amount of drug transported amongst all the formulations. Chitosan is a well known absorption enhancer and acts by opening the tight junctions in the epithelial cells of the everted sac thus permitting more drug to pass through (22). Because of their positive charge, chitosan can interact with anionic components of the glycoproteins on the surface of epithelial cells. It has also been suggested that cationic macromolecules are able to displace cations from electronegative sites (such as tight junctions) on a membrane which require coordination with cations with dimensional stability. In addition, it has been shown that the interior of the tight junction channels (pores) are highly hydrated and contain fixed negative charges. The relative concentration of specific type of ions within the pores could result in substantial alterations in tight junction resistance leading to loosening or opening of the pore. Another study confirming the nature of chitosan as an absorption enhancer was performed by Schipper et al., (23) where it was found that the positive charge of the chitosan polymer makes possible a charge interaction between the chitosan and the negatively charged surface of the epithelial cell membrane.

Storage stability studies

All tablet formulations (F1-F3) were subject to stability studies to evaluate the degradation of the tablet formulations after storage of 3 mo. Figure 7 shows the drug release profiles after one, two and three months of storage (stored at 40°C/ 75% RH).

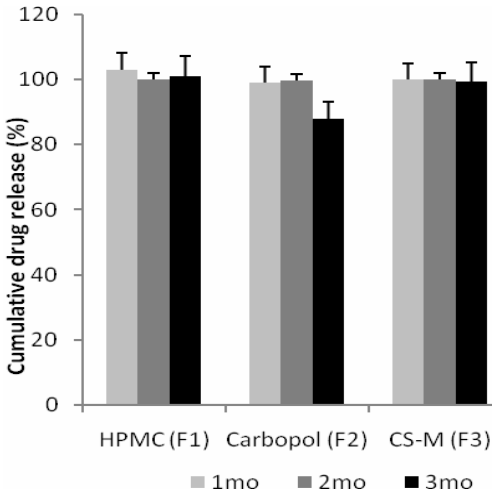

Figure 7 Cumulative niacinamide drug release from tablets containing 20% concentrations of HPMC K4M (F1), Carbopol 974P-NF (F2) and CS-M (F3).

As seen from Figure 7, after 3 mo. of storage there were no significant differences in release rates of niacinamide from any of the formulations. Formulation (F2) containing Carbopol 974P-NF® (F2) showed a slight decrease in drug release after 3 months of storage however the difference was insignificant compared to other polymers. CS-M formulation (F3) also demonstrated a stable profile indicating that no drug degradation was apparent. Overall, all polymer formulations appeared to be stable in terms of drug release for the duration of the 3 mo study.

Conclusion

In conclusion, we successfully developed a novel chitosan hydrogel salt complex polymer using a freeze-drying method thus providing a good alternative to the use of parent chitosan with its inherent problems in formulation development. When compared to conventional polymers such as HPMC K4M and Carbopol 974P-NF ®, the modified chitosan compound demonstrated better characteristics in terms of swelling properties, *in vitro* drug release from the polymer and transport of drug across the intestine. In terms of bioadhesion and stability, the modified chitosan CS-M showed similar characteristics to its commercial counterparts. Overall, the modified chitosan polymer exhibited better qualities than its parent compound, chitosan, and thus may be of use to create controlled release formulations.

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